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Informative value of the *mrjp3* locus for assessing hybridisation and productivity of bee colonies

Информативность локуса *mrjp3* для оценки гибридизации и продуктивности пчелиных семей

N.V. Ostroverkhova, S.A. Rosseykina
Н.В. Островерхова, С.А. Россейкина

The National Research Tomsk State University, Prosp. Lenina 36, Tomsk 634050 Russia. E-mail: nvostrov@mail.ru, rosseykina75@mail.ru
Национальный исследовательский Томский государственный университет, просп. Ленина 36, Томск 634050 Россия.

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Ключевые слова: медоносная пчела, *Apis mellifera,* подвиды медоносной пчелы, микросателлитный локус *mrjp3*, гибридизация пчёл, маточное молочко, продуктивность.

Abstract. The honeybee *Apis mellifera* (Linnaeus, 1758) is of enormous ecological and economic importance. Currently, the intensification of bee product production and increasing the efficiency of pollination of agricultural plants is impossible without the use of genomic selection in beekeeping. A priority task is the development of diagnostic molecular genetic markers associated with selectively significant traits in bee breeding.

The microsatellite *mrjp3* locus, located in the coding region of the *mrjp3* gene encoding the main proteins of royal jelly in honeybees, is considered as a promising DNA marker for molecular selection. The microsatellite *mrjp3* locus differentiates bee subspecies (diagnostic alleles specific to the M and C lineages have been identified) and allows for the determination of the degree of hybridisation in colonies of southern origin based on the combined frequency of alleles specific to the C lineage (alleles of 406 bp and 520 bp). An analysis of the association between royal jelly productivity and the degree of hybridisation in bee colonies revealed that with increasing hybridisation (purebred colonies → colonies with traces of hybridisation → hybrids), royal jelly productivity decreases. Using M lineage colonies, it was revealed that, in addition to purebred colonies, high genetic diversity of the *mrjp3* locus and a representation of alleles specific to the dark forest bee (alleles of 390 bp, 435 bp, and 510 bp) are necessary conditions for high royal jelly productivity in bee colonies. The possibility of using the microsatellite *mrjp3* locus as a marker of royal jelly productivity in honeybee breeding is discussed.

Резюме. Медоносная пчела *Apis mellifera* (Linnaeus, 1758) имеет огромное экологическое и экономическое значение. В настоящее время интенсификация производства пчелопродуктов, повышение эффективности опыления сельскохозяйственных растений невозможно без внедрения в практику пчеловодства методов геномной селекции. Первоочередной задачей является разработка диагностических молекулярно-генетических маркеров, ассоциированных с селективно значимыми признаками, для использования в селекционно-племенной работе с медоносными пчёлами.

Микросателлитный локус *mrjp3,* расположенный в кодирующей части гена *mrjp3*, отвечающего за производство основных белков маточного молочка у пчёл, рассматривается как перспективный ДНК-маркер для молекулярной селекции. Локус *mrjp3* дифференцирует подвиды медоносной пчелы (выявлены диагностические аллели, специфичные для подвидов эволюционных линий M и C), а также позволяет определить степень гибридизации семей южного происхождения по сочетанию и суммарной частоте аллелей, специфичных для линии C (аллели размером 406 пн и 520 пн). Анализ ассоциации между показателями продуктивности маточного молочка семей и их степенью гибридизации показал, что при повышении степени гибридизации (чистопородные семьи → семьи со следами гибридизации → гибриды) продуктивность маточного молочка семьи снижается. На примере пчелиных семей линии M выявлено, что кроме чистопородности необходимыми условиями высокой продуктивности семей по маточному молочку является значительное генетическое разнообразие локуса *mrjp3* и представленность аллелей локуса, специфичных для тёмной лесной пчелы (аллели размером 390 пн, 435 пн и 510 пн). Обсуждается возможность использования микросателлитного локуса *mrjp3* как маркера продуктивности маточного молочка в селекции медоносной пчелы.

Introduction

Currently, new technologies and molecular genetic approaches are actively used in the breeding of farm animals [Bourgeois, Beaman, 2017; Stolpovsky et al., 2020; Kalashnikov et al., 2021; Avalos, Bilodeau, 2022; Kaskinova et al., 2023]. The biology and genetics of the honeybee *Apis mellifera* Linnaeus, 1758, such as its social lifestyle, polyandry, parthenogenesis, etc., complicate the development of genomic selection in beekeeping [Zhou et al., 2026]. In honeybees, genetic

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markers associated with biological and economic traits remain poorly developed [Yunusbaev et al., 2019; Ostroverkhova, 2024].

The *mrjps* family genes (*mrjp1-mrjp9*), encoding the Major Royal Jelly Proteins (MRJPs), are among the first genes for which associations of some polymorphic variants with economic traits in honeybees have been assessed [Baitala et al., 2010; Parpinelli et al., 2014]. In Africanised bees of the Brazilian population, high polymorphism of three *mrjps* genes (*mrjp3*, *mrjp5,* and *mrjp8*) was detected; some alleles of these genes are associated with royal jelly productivity.

Of particular interest is the *mrjp3* gene. The highly repetitive region is located in the 3′-coding region of the *mrjp3* gene; it is the VNTR region and is designated as microsatellite *mrjp3* locus [Albert et al., 1999]. The sequence of the repetitive region consists of two distinguishable segments formed by 15-nucleotide repeats. The first segment located more 5'-terminally is more conserved and includes 6–8 copies of the repeating sequence. The second segment is less conserved. The copy number of this segment varies from 5 to 21 [Albert et al., 1999; Albertova et al., 2005].

In the honeybee *A. mellifera*, more than 10 *mrjp3* different alleles ranging from 380 bp to 610 bp in their size have been identified [Albert et al., 1999; Baitala et al., 2010; Parpinelli et al., 2014; Ostroverkhova et al., 2018a, b]. In the Carniolan honeybee *Apis mellifera carnica* Pollmann, 1879 (evolutionary lineage C), living in Germany, five alleles (*Mrjp3a*, *Mrjp3b*, *Mrjp3c*, *Mrjp3d,* and *Mrjp3e*) have been identified [Albert et al., 1999]. In the dark forest bee *Apis mellifera mellifera* L., 1758 (evolutionary lineage M), living in Siberia, we previously identified 8 alleles; the 530 bp allele was dominant with a frequency of more than 75 % [Ostroverkhova et al., 2018b]. Interestingly, honeybees of southern origin (lineage C), such subspecies as *A. m. carnica*, *Apis mellifera ligustica*, Spinola, 1806, as well as *Apis mellifera carpathica* — a branch of *A. m. carnica*, cultivated in the central and southern regions of Russia, had specific alleles of 406 bp and 520 bp in size (frequency 40–50 % and 20 %, respectively). In bees of the C lineage subspecies, the 530 allele, specific to the M lineage subspecies, was rare (frequency less than 5 %). In honeybee *A. m. mellifera*, alleles specific to C lineage subspecies were detected with low frequency (the frequency of the 406 allele is 3 %; the 520 allele was not detected) [Ostroverkhova et al., 2018a]. The microsatellite *mrjp3* locus can be considered as a DNA marker differentiating subspecies of different evolutionary lineages (M and C).

Using data on the variability of the microsatellite *mrjp3* locus for Africanised bee colonies, associations of the alleles C, D, and E with royal jelly productivity were identified [Baitala et al., 2010; Parpinelli et al., 2014].

Considering the importance of selection and breeding work with bee colonies in those natural and climatic conditions where honeybee breeding is planned [Büchler et al., 2010; Kovačić et al., 2020; Ostroverkhova et al., 2024], as well as the need for careful use of data obtained in other geographic regions, the aim of this work was to assess the informativeness of the microsatellite *mrjp3* locus as a DNA marker of royal jelly productivity and/or the degree of hybridisation of bee colonies for molecular selection in Siberian conditions. For the first time, the *mrjp3* locus was used to characterize the hybridisation of honeybees of different evolutionary lineages and to identify associations of the locus alleles with royal jelly productivity in European bee subspecies.

Materials and methods

The research was carried out at two isolated apiaries of the Tomsk State University, located in the Tomsk region (Kornilovo village area, N56.495628°, E85.170431° and the vicinity of the city of Tomsk, N56.4977°, E84.9744°). At the apiary of Kornilovo, the dark forest bee *A. m. mellifera* (lineage M) is cultivated; at the second apiary, the descendants of the Carpathian bee (*A. m. carpathica*) colonies obtained from the bee nursery (lineage C) are cultivated. The assignment of bee colonies to an evolutionary lineage was determined based on mtDNA analysis data. Two samples were formed based on the maternal lineage of the bee colonies: 6 colonies belonging to the M lineage and 7 colonies of southern origin (lineage C). In total, samples of worker bees obtained from 13 colonies belonging to different evolutionary lineages (M and C) were analysed.

Initially, the origin of bee colonies was determined, then the genetic diversity of the microsatellite *mrjp3* locus was studied in bees of these colonies and an assessment of the associations between the identified alleles of the *mrjp3* locus, the hybridisation, and royal jelly productivity was carried out.

Assessment of the origin of the bee colony. Honeybees were analysed by the following subspecies discrimination methods: mtDNA and morphometric analyses. To determine the maternal origin of the bee colony, variability in the *COI-COII* mtDNA locus was studied (three individuals from the colony were analysed). The PQQ variant of the *COI-COII* locus in honeybees indicates that the colony is maternally descended from *A. m. mellifera* (lineage M), whereas the Q variant of the *COI-COII* locus indicates that it is descended from a subspecies of southern origin (lineage C).

Morphometric measurements of the bee samples included the following main wing parameters (wing venation): the cubital index, the hantel index, and the discoidal shift [Konusova et al., 2016]. At least 25 individuals from a colony were analyzed. Morphometric data was expressed in mean ± standard error of the mean (M ± m). The morphometric parameters obtained were compared with the values of standards adopted for worker bees of different bee subspecies [Cauia et al., 2008]. If the morphometric parameters of bees correspond to the dark forest bee's standard, this colony was considered potentially "pure" *A. m. mellifera*. If the morphometric parameters of bees were not consistent with *A. m. mellifera* breed standards, we considered bee colonies as hybrids between the dark forest bee (*A. m. mellifera*) and the Carpathian bee (*A. m. carpathica*).

Molecular genetic analysis. Total DNA was extracted from the thorax of worker bees using the DNA-Extran reagent kit (Synthol, Russia) and analyzed by polymerase chain reaction (PCR) using the BioMaster HS-Taq PCR-Color kit (Biolabmix, Russia).

To amplify the *COI-COII* mtDNA locus, the following primers were used: F-5' — CACATTTAGAAATTCCATTA, R-5' — ATAAATATGAATCATGTGGA. PCR included an initial denaturation of 5 min at 95 °C, followed by 35 cycles of 95 °C for 1 min, 57 °C for 2 min and 72 °C for 2 min with a final extension of 7 min at 72 °C [Nikonorov et al., 1998]. The size of the amplified DNA fragments was determined using a 1.5 % agarose gel. The results were documented with the use of Gel-Doc XR+ (Bio-Rad Laboratories, Inc., USA).

To amplify the microsatellite *mrjp3* locus, the following primer sequences were used: F-5′[FAM] — ATGTAATTTTGAAGAATGAACTTG, R-5′ — TGTAGATGACTTAATGAGAAACAC (one primer with a fluorescent label). PCR included an initial denaturation of 2 min at 94 °C, followed by 30 cycles of 94 °C for 30 sec, 54 °C for 30 sec and 72 °C for 1 min with a final extension of 7 min at 72 °C [Albert et al., 1999]. From 17 to 31 individuals from each colony were studied. Samples were analysed on an ABI Prism 3730 genetic analyser using the GeneMapper Software (Applied Biosystems, Inc., United States) at the Medical Genomics Collective Use Center (Research Institute of Medical Genetics, Tomsk Research Medical Center, Russian Academy of Sciences). Statistical data treatment (calculation of allele frequencies, standard error) was performed using the GenAlEx 6.5 software program [Peakall, Smouse, 2006].

To assess the associations between alleles (their combinations) of the *mrjp3* locus and royal jelly productivity, the Spearman rank correlation method (*r*s) was used. Statistical data processing was performed in Statistica 12 (StatSoft Inc., Tulsa, OK, USA).

Conflict of interest. The authors declare that they have no potential conflict of interest in relation to the present study.

The present work is registered in ZooBank (www.zoobank.org) under urn:lsid:zoobank.org:pub:3559D135-ED9B-49DC-8803-2F8CBE7B4AFC

Results and discussion

Characterisation of the origin of the bee colonies

According to the analysis of variability of the *COI-COII* mtDNA locus, two samples of bee colonies belonging to different evolutionary lineages (M and C) were formed (Table 1). The M lineage sample of bees included four colonies that corresponded to the dark forest bee standard, and two colonies that did not correspond to the *A. m. mellifera* standard in morphometric parameters and were hybrids between *A. m. mellifera* and *A. m. carpathica*.

The C lineage sample of bees included seven colonies. According to morphometric analysis, these colonies did not correspond to any subspecies of southern origin and were hybrids between *A. m. carpathica* and *A. m mellifera*. The degree of hybridisation of bee colonies varied considerably, ranging from traces of hybridisation (colony 2; bees did not correspond to the *A. m. carpathica* standard only for the "discoidal shift" trait) to a highly hybridised colonies (colony 1; for most of the studied morphometric parameters, the bees showed a deviation from the standards accepted for subspecies of southern origin).

Variability of the *mrjp3* locus in bees of different evolutionary lineages

The results of the present study on *mrjp3* locus variability in bees of different origins are consistent with our earlier findings on locus polymorphism in Siberian bee populations [Ostroverkhova et al., 2018a]. In bees from pure-bred colonies, as well as in hybrids based on *A. m. mellifera* (M lineage), the predominant allele was 530 bp in size, with a frequency ranging from 50 to 100 % (Table 2).

In hybrid colonies belonging to the C lineage, alleles of 406 bp and 520 bp specific for subspecies of C lineage were identified, as well as an 530 bp allele specific to *A. m. mellifera*, indicating hybridisation of bees. Therefore, the microsatellite *mrjp3* locus should be considered not only as a DNA marker differentiating subspecies of the M and C lineages, but also as a marker of hybridisation.

Variability of the *mrjp3* locus and hybridisation

To assess the degree of hybridisation in bee colonies originating from southern subspecies (C lineage), the representation of alleles specific to the C lineage (frequency of alleles of 406 bp and 520 bp) was determined (Table 2). Based on the combined frequency of these alleles (406 + 520), the colonies were ranked as follows: colony 7 (67.2 %) — colony 2 (60.0 %) — colony 4 (45.2 %) — colony 6 (36.7 %) — colony 5 (36.6 %) — colony 3 (30.0 %) — colony 1 (13.3 %). This sequence reflects the degree of hybridisation, ranging from colonies with only minor traces of hybridisation (colony 7) to highly hybridised colonies (colony 1).

For hybrid colonies, the results of microsatellite analysis and morphometric data are consistent (Table 1). In particular, the "discoidal shift" trait, which is one of the first morphometric parameters to deviate from the subspecies standard during bee hybridisation, changes as follows: the proportion of individuals with a positive discoidal shift, specific to southern subspecies, decreases, and the number of individuals with zero or negative discoidal shift increases.

For example, weakly hybridised colonies 7 and 2 showed the lowest proportion of bees with a negative discoidal shift characteristic of the dark forest bee (9 % and 14.3 %, respectively). Colony 2 had the highest proportion of individuals with a positive discoidal shift

Table 1. Characteristics of morphometric parameters and variants of the *COI-COII* mtDNA locus in bees of the studied colonies
Таблица 1. Характеристика морфометрических показателей и вариантов локуса *COI-COII* мтДНК у пчёл изученных семей

Bee colony, №	Variant of the locus *COI-COII*	Cubital index, standard units		Hantel index, standard units		Discoidal shift, %			Origin of the bee colony
		Lim: min-max	*M ± m*	*Lim: min-max*	*M ± m*	–	0	+	
Bee colonies descended from the dark forest bee (evolutionary lineage M)									
1	PQQ	1.30–2.00	1.75 ± 0.04	0.720–0.871	0.811 ± 0.010	100.0	0	0	*A. m. mellifera*
2	PQQ	1.32–1.82	1.53 ± 0.03	0.722–0.903	0.802 ± 0.010	97.0	3.0	0	*A. m. mellifera*
3	PQQ	1.20–1.67	1.45 ± 0.02	0.723–0.900	0.837 ± 0.009	97.0	3.0	0	*A. m. mellifera*
4	PQQ	1.12–1.76	1.51 ± 0.03	0.758–0.919	0.845 ± 0.008	93.0	7.0	0	*A. m. mellifera*
5	PQQ	1.67–3.00	2.23 ± 0.12	0.882–1.107	0.984 ± 0.023	10.0	10.0	80.0	Hybrid
6	PQQ	1.45–2.73	1.87 ± 0.06	0.743–1.033	0.857 ± 0.013	55.6	40.7	3.7	Hybrid
Bee colonies of southern origin (evolutionary lineage C)									
1	Q	1.70–2.90	2.30 ± 0.06	0.790–1.065	0.917 ± 0.012	36.7	53.3	10.0	Hybrid
2	Q	1.80–3.20	2.69 ± 0.07	0.852–1.075	0.990 ± 0.012	14.3	50.0	35.7	Hybrid
3	Q	2.10–3.90	2.83 ± 0.08	0.743–1.000	0.880 ± 0.013	23.9	50.0	26.1	Hybrid
4	Q	1.75–3.20	2.39 ± 0.06	0.714–1.000	0.870 ± 0.010	45.2	48.4	6.4	Hybrid
5	Q	1.90–3.90	2.30 ± 0.06	0.719–1.037	0.847 ± 0.012	60.0	30.0	10.0	Hybrid
6	Q	1.90–3.00	2.36 ± 0.07	0.794–1.188	0.932 ± 0.030	31.0	62.0	7.0	Hybrid
7	Q	1.81–2.90	2.00 ± 0.05	0.866–1.020	0.937 ± 0.012	9.0	90.7	0.3	Hybrid
Standard for *A. mellifera*	PQQ	1.30–1.90	1.5–1.7	0.600–0.923	No data	91–100	5–10	0	*A. m. mellifera*
	Q	2.40–3.00	2.70	0.925–1.200	No data	0–5	0–20	80–100	*A. m. carnica*
	Q	2.30–3.00	2.60	0.920–1.200	No data	0	5	95	*A. m. carpathica*

Note. *Lim* — limits of the trait value; *M*±*m* — mean value of a trait ± error.

Примечание. *Lim* — пределы значения признака; *M*±*m* — среднее значение признака ± ошибка.

(35.7 %). In contrast, strongly hybridised colonies 1, 3, and 5 had many bees with negative or zero discoidal shift (74–90 %). Colonies 4 and 6, with intermediate levels of hybridisation, had intermediate numbers of bees with zero or negative discoidal shift (31–62 %).

For all colonies belonging to the M lineage, the predominant allele among the studied bees was the 530 bp allele specific to *A. m. mellifera*. In hybrid bees, the 520 bp allele, characteristic of bees of southern origin, was also identified (Table 2).

Thus, the microsatellite *mrjp3* locus represents an informative molecular marker for assessing the degree of bee hybridisation. This is particularly important today, as honeybee populations are undergoing massive hybridisation, accompanied by the loss of purebred subspecies [Muñoz et al., 2015; Henriques et al., 2018a; Oleksa et al., 2023; Knoll et al., 2024]. Combined with morphometric analysis, molecular markers provide a powerful tool for studying the genetic processes underlying bee hybridisation, especially bee subspecies belonging to different evolutionary lineages. Such approaches also make it possible to investigate the replacement of genes from one subspecies by those of another, estimate the proportion of "pure-bred" and introgressed genes, and determine when introgression processes began. Mitochondrial DNA loci are widely used to assess introgression [Muñoz, De la Rúa, 2021; Oleksa et al., 2021; Zhou et al., 2026]. Analysis of nuclear DNA markers using panels of 8–10 or more DNA loci (microsatellites or SNPs) [Péntek-Zakar et al., 2015; Muñoz et al., 2017; Ilyasov et al., 2020; Yumaguzhin et al., 2022] or SNP array technologies [Pinto et al., 2014; Muñoz et al., 2015; Lukic et al., 2024] requires specialized equipment and substantial financial resources. The development of informative DNA markers such as the microsatellite locus *mrjp3* could therefore enable cost-effective genetic studies.

It is known that bee hybridisation may proceed differently and alter the subspecies composition of honeybees of different evolutionary lineages. For example, weak introgression was shown between the subspecies *Apis mellifera iberiensis* Engel, 1999 (lineage M) and subspecies of lineage C (*A. m. ligustica*, *Apis mellifera cecropia* Kiesenwetter, 1860, *Apis mellifera macedonica* Ruttner, 1988, and *A. m. carnica*). Conversely, for another subspecies of lineage M (*A. m. mellifera*), a significant level of introgression with subspecies of lineage C was detected [De la Rúa et al., 2009; Soland-Reckeweg et al., 2009; Oleksa et al., 2011; Pinto et al., 2014; Muñoz et al., 2015; Henriques et al., 2018b; Parejo et al., 2018]. In addition, an assessment of hybridisation level is essential for the identification and monitoring of purebred bee colonies and populations, which is crucial for the preservation and restoration of the unique gene pools of native bees, such as the dark forest bee [Jensen et al., 2005; Ilyasov et al., 2016, 2020; Hassett et al., 2018; Kaskinova et al., 2021; Yumaguzhin et al., 2022; Ostroverkhova et al., 2024; Frunze et al., 2025].

It is known that hybridisation has a negative impact on the economic traits of bee colonies, including produc-

Table 2. Variability of the *mrjp3* microsatellite locus in honeybees of different origins (lineages M and C)
Таблица 2. Вариабельность микросателлитного локуса *mrjp3* у пчёл разного происхождения (линии М и С)

Bee colony, №	Allele size of the *mrjp3* locus, bp									Total allele frequency, %
	390	406	435	465	485	495	500	510	520	530	
Bee colonies descended from the dark forest bee (evolutionary lineage M)											390+435+510
1	0	0	0	0	0	0	0	0	0	1.000±0.000	0
2	0.077±0.679	0	0.077±0.679	0	0.038±0.490	0	0	0.077±0.679	0	0.731±1.131	23.1
3	0.114±1.144	0	0.091±1.211	0.068±1.353	0	0.068±1.311	0	0	0	0.659±0.408	20.5
4	0.065±0.967	0.032±0.696	0	0	0.032±0.696	0.065±0.496	0	0.306±1.815	0	0.500±1.969	37.1
5	0	0	0	0.059±0.654	0.059±0.654	0.147±0.401	0	0	0.176±0.357	0.559±0.199	0
6	0.069±0.033	0	0	0	0.293±0.060	0	0	0	0.052±0.029	0.586±0.065	6.9
Bee colonies of southern origin (evolutionary lineage C)											406+520
1	0	0	0	0.217±0.053	0.400±0.063	0	0.083±0.036	0	0.133±0.044	0.167±0.048	13.3
2	0.050±0.028	0.083±0.036	0	0	0.100±0.039	0.250±0.056	0	0	0.517±0.065	0	60.0
3	0	0.200±0.052	0	0	0.300±0.059	0.300±0.059	0	0	0.100±0.039	0.100±0.039	30.0
4	0	0.081±0.035	0	0	0.468±0.063	0	0	0	0.371±0.061	0.065±0.031	45.2
5	0	0.083±0.036	0	0	0.383±0.063	0	0	0	0.283±0.058	0.183±0.050	36.6
6	0.383±0.063	0	0	0	0.083±0.036	0	0	0	0.367±0.062	0.117±0.041	36.7
7	0.138±0.045	0.379±0.064	0	0	0	0	0	0	0.293±0.060	0.155±0.048	67.2

Note. The *mrjp3* locus allele frequency is shown (with an error). For bee colonies of lineage M, the combined frequency of alleles specific to the dark forest bee is shown, and for bee colonies of lineage C, the combined frequency of alleles specific to subspecies of southern origin is shown.

Примечание. Приведена частота аллелей локуса *mrjp3* (с ошибкой). Для семей линии М приведена суммарная частота аллелей, специфичных для тёмной лесной пчелы, для семей линии С — аллелей, специфичных для подвидов южного происхождения.

tivity [De la Rúa et al., 2009; Kovačić et al., 2020]. Therefore, it is of considerable interest to assess the association between the variability of the microsatellite *mrjp3* locus and royal jelly productivity in bee colonies of different origins (M and C lineages).

Associations between alleles of the microsatellite *mrjp3* locus and royal jelly productivity in honeybee colonies

An assessment of the association between alleles of the microsatellite *mrjp3* locus and the royal jelly productivity of honeybee colonies was carried out (Table 3).

In hybrid colonies (lineage C), royal jelly productivity was statistically significantly associated with the combined frequency of two alleles (406 bp and 520 bp) characteristic of subspecies of southern origin (rs = 0.857, $p < 0.05$). The most productive colony 7 (552 mg of royal jelly per queen cell) had the highest combined frequency of the 406 bp and 520 bp alleles (67.2 %) and was weakly hybridised. In contrast, the less productive colony 1 (230 mg of royal jelly per queen cell) had the lowest combined frequency of the two "southern" alleles, equal to 13.3 %, i.e., it had a high degree of hybridisation. Therefore, the level of royal jelly productivity can be determined by the presence of 406 bp and 520 bp alleles and by the degree of hybridisation (replacement of genetic variants characteristic of southern subspecies).

For bee colonies originating from the dark forest bee (lineage M), no statistically significant association was found between the predominant 530 bp allele and royal jelly production (rs = 0.086, $p > 0.05$). The presence of the 530 bp allele probably does not indicate productivity, but rather the origin of the bee colony (an allele specific to the dark forest bee). The 530 bp allele, which is predominant in dark forest bees of Siberian populations, is probably necessary for adaptation to harsh natural and climatic conditions (survival of bees in Siberian conditions), but is insufficient for maintaining high economic performance. It is important to note that hybrid colonies, such as colonies 5 and

Table 3. Royal jelly productivity of bee colonies of different lineages (M and C)
Таблица 3. Продуктивность пчелиных семей разных линий (М и С) по маточному молочку

Lineage M			Lineage C		
Bee colony, №	Royal jelly productivity		Bee colony, №	Royal jelly productivity	
	Yield of royal jelly from the queen cell, mg*	*Lim: min-max*		Yield of royal jellyfrom the queen cell, mg*	*Lim: min-max*
4	594.70±62.42	285–854	7	552.0±55.73	390–630
2	351.67±84.31	231–514	2	401.1±27.61	210–490
3	334.00±93.95	100–556	4	445.2±26.40	290–600
1	452.80±80.20	263–728	6	430.0±30.00	310–595
Hybrid bee colonies			5	239.7±69.80	180–615
6	271.80±27.70	250–650	3	258.2±36.50	190–320
5	286.80±30.50	114–513	1	230.0±37.61	150–350

Note. Bee colonies are arranged according to the representation of specific alleles of the *mrjp3* locus (in descending order): for lineage M — the total frequency of alleles of 390, 435, and 530 bp; for lineage C — the alleles of 406 and 520 bp (see Table 2). Within the lineage M, pure-bred and hybrid colonies are presented separately. Designations: *min* — the minimum amount of royal jelly in one queen cell; *max* — the maximum amount of royal jelly in one queen cell; * — the average value of royal jelly yield from a queen cell per colony (±error) is presented.

Примечание. Семьи расположены в соответствии с представленностью специфических аллелей локуса *mrjp3* (по убыванию): для линии М — суммарная частота аллелей 390, 435 и 530 пн; для линии С — аллелей 406 и 520 пн (см. Табл. 2). В группе семей линии М отдельно представлены чистопородные и гибридные семьи. Обозначения: *min* — минимальное количество маточного молочка в одном маточнике; *max* — максимальное количество маточного молочка в одном маточнике; * — представлено среднее значение выхода маточного молочка из маточника на семью (±ошибка).

6, are characterised by the lowest royal jelly production (272–287 mg). Pure-bred dark forest bee colonies (colonies 1–4) demonstrated high royal jelly productivity (334–595 mg), and the statistical significance of the combined frequency of the 390 bp, 435 bp, and 530 bp alleles, specific for *A. m. mellifera*, for the royal jelly productivity of bee colonies was shown (rs = 0.800, $p < 0.05$). Therefore, the diversity of the *mrjp3* locus may contribute to colony productivity, but is probably not the only indicator determining the economic traits of bee colonies.

Our results are partially consistent with the data obtained for Africanised honeybee colonies [Baitala et al., 2010]. It was shown that Africanised bees with heterozygous genotypes DE, DC, and EC of the *mrjp3* locus were the most productive. Despite the accumulation of homozygous genotypes at the *mrjp3* locus during the selection of highly royal jelly productive colonies, a high total heterozygosity at the *mrjps* loci was shown [Parpinelli et al., 2014]. This suggests that not only specific genetic variants, but also the overall genetic diversity of bees are significant for colony productivity.

In *A. m. ligustica* bees, significant genetic diversity and a high level of heterozygosity at microsatellite loci have been established [Chen et al., 2005]. Furthermore, strong genetic differentiation has been demonstrated both between highly productive *A. m. ligustica* lines from different regions of China and between different populations, regardless of their productivity [Ji et al., 2009; Yin et al., 2011; Cao et al., 2016].

Although the microsatellite *mrjp3* locus and other genes of the *mrjps* family are considered promising tools for genetic studies of the honey bee (e.g., *A. mellifera* taxonomy) and genomic selection, the development of informative DNA markers remains an important issue in beekeeping. The combination of the sequenced *A. mellifera* genome with high-density SNP array technologies could greatly accelerate the identification of significant genetic polymorphisms, including those associated with royal jelly productivity. For example, a comparative analysis of differentially expressed genes (DEGs) in nurse bees from colonies differing in royal jelly productivity, conducted using a microarray containing more than 10,000 genes, found three genes (dopamine receptor Dop2, signal sequence receptor beta SsRbeta, and hexamerin 70a Hex 70a) associated with high royal jelly production [Cao et al., 2016]. In another study aimed at identifying DEGs associated with changes in gene expression levels in bees differing in royal jelly productivity, DNA microarray analysis of 11,689 genes identified 369 DEGs associated with royal jelly content in honey bees [Nie et al., 2017]. Among these, 201 DEGs (54.47 %) showed increased expression, while 168 DEGs (45.53 %) exhibited decreased expression in bees with high royal jelly production. These findings may be useful for identifying QTL loci involved in controlling this trait and provide a genetic foundation for further investigation of the molecular mechanisms underlying elevated royal jelly production in honey bees.

Conclusions

Based on the analysis of variability in the microsatellite *mrjp3* locus, the assessment of hybridisation levels, and the royal jelly productivity of honeybee colonies of different origins, several conclusions can be drawn. First, the microsatellite *mrjp* locus may be regarded as a DNA marker whose alleles differentiate honeybee subspecies belonging to the M and C evolutionary lineages. Second, the *mrjp3* locus appears to be informative for determining the degree of hybridisation in honeybee colonies, particularly in colonies of the C lineage. Finally, the *mrjp3* locus may be used to evaluate the royal jelly productivity of honeybee colonies. However, several factors should be considered, including the genetic purity of the colony, the level of genetic diversity at the locus,

and the specific combination of locus-specific alleles. At the same time, further studies are needed to assess the genetic diversity of honeybee subspecies and colonies with different economically important traits, as well as the influence of environmental factors on the genetic diversity of bee subspecies and colonies differing in productivity. These findings are especially important for the development of selective breeding programs aimed at producing honeybee colonies with genetic characteristics associated with high royal jelly productivity.

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